Exposure to Sidestream Cigarette Smoke Alters Bronchiolar Epithelial Cell Differentiation in the Postnatal Rat Lung

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This study examines the effects of an ambient concentration of aged and diluted sidestream cigarette smoke (ADSS) on bronchiolar epithelial cell development and the expression of cytochrome P450 isozyme 1A1 protein in the postnatal rat lung. In control animals, the labeling indices for epithelial cells in proximal bronchi and terminal bronchioles at 7 days of age were 2.3 and 4.9%, respectively, and decreased to 0.1 and 0%, by 100 days of age. With exposure to ADSS from birth, the labeling index of epithelial cells in distal airways of rats was significantly reduced at 7 and 14 days of age, but not in epithelial cells of proximal bronchi. The expression of P450 isozyme 1A1 antigen in bronchiolar epithelial cells of control rats reached the maximal level observed at 21 days of age and subsequently decreased to low levels at 50 and 100 days of age. In contrast, exposure to ADSS significantly increased the distribution and intensity of staining for 1A1 antigen in bronchiolar epithelial cells of proximal and distal airways as early as 7 days of age and maintained elevated levels of 1A1 protein in these cells through 100 days of age. At 21 and 50 days of age, NADPH reductase protein expression was higher in the airway epithelium of rats exposed from birth to ADSS than that noted in the airways of controls. In contrast, cytochrome P450 isozyme 2B and Clara cell secretory protein expression were unchanged. We conclude that in postnatal rats, exposure to ADSS from birth (*1*) inhibits cell kinetic activity in epithelial cells of terminal bronchioles but not in epithelial cells of the proximal bronchi, (*2*) accelerates and maintains expression of cytochrome P450 isozyme 1A1 protein in Clara cells during postnatal lung development, (*3*) increases bronchiolar expression of NADPH reductase but does not increase expression of cytochrome P450 isozyme 2B or Clara cell secretory protein, and (*4*) alters airway epithelial cell development.

The mammalian lung is extremely sensitive to a large number of inhaled toxicants (1). Many such toxicants are found in environmental tobacco smoke (ETS), which is a combination of exhaled mainstream smoke and sidestream smoke emitted from the smoldering end of a cigarette. A strong relationship has been demonstrated between respiratory illness in young children and ETS exposure; likewise, an increased risk of lung cancer has been noted in adults who have had significant past exposure to ETS (2–5). However, the cellular mechanisms responsible for these diseases are poorly understood. Recent studies in rats using aged and diluted sidestream smoke (ADSS) at concentrations similar to or slightly above those found in the environment have not shown any association between ADSS exposure and pathologic changes in the lung (6, 7). However, these studies focused on the mature lung and did not examine the effects of ADSS in neonatal animals in which the lungs undergo rapid growth and cellular differentiation.

One factor that is likely to play a key role in modulating the effects of inhaled xenobiotics is the cytochrome P450 system. This system is critical in the bioactivation of a number of procarcinogenic substrates, including aromatic amines and polycyclic hydrocarbons (8–10) present in sidestream smoke. Among the 40 cell types in the lung (11), the nonciliated bronchiolar epithelial (Clara) cell contains high levels of P450 monooxygenases (12) and therefore is a target of many inhaled procarcinogenic substrates via the generation of reactive (and toxic) metabolites within these same cells.

Of the more than 40 different cytochrome P450 isoforms (13), isozyme 1A1 is probably the most important in interactions with components of cigarette smoke, such as benzo(a)pyrene, a potent inducer of 1A1. Isozyme 1A1 has been found throughout the respiratory system and is present in Clara cells of normal rats (14). Studies in the rabbit have shown that the P450 monooxygenase system develops postnatally. In rabbits younger than 2 days of age, P450 activity is undetectable but attains adult levels by 28 days of age (15).

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Abbreviations: aged and diluted sidestream smoke, ADSS; Clara cell 10 kD protein, CC10; environmental tobacco smoke, ETS; tritiated thymidine, [^{3}H]TdR; labeling index, LI; phosphate-buffered saline, PBS; 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, TCDD; total suspended particulates, TSP.

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Whether exposure to substrates that serve as inducers has any effect on this developing enzyme system during the period of postnatal development is not known.

In addition to containing cytochrome P450 monooxygenases, the Clara cell also serves as the stem cell for Clara and ciliated cells of the lower airways, since basal cells are typically absent in the more distal generations of the tracheobronchial tree (16). The progenitor role of the Clara cell has been demonstrated in adult animals of several species (16–22). Unlike the adult lung, which has very low kinetic activity, fetal lungs have very high kinetic activity (labeling index [LI]: 6%), due in large measure to the progenitor role of the Clara cell (23). However, little is known regarding airway epithelial cell kinetics in neonates.

This study addresses whether sidestream smoke at a concentration that could be encountered indoors affects the nonciliated bronchiolar epithelial (Clara) cell, a lung cell type that differentiates postnatally and is a primary target for inhaled pulmonary toxicants. The rat was selected for these studies based on the marked growth and development of the lungs in this species during the first months of life. In addition, an extensive literature is also available on pulmonary response of rats to environmental toxicants (24–27). Our primary goals were to examine the impact of ADSS on a variety of functions of Clara cells, including proliferation, maturation, differentiation, and cytochrome P450 isozyme 1A1 expression during critical periods of postnatal development and growth.

Materials and Methods

Animals

Timed-pregnant, specific pathogen-free Sprague-Dawley rats were shipped from Zivic-Miller Laboratories, Inc. (Zelienople, PA) to our institution 2 days after mating. The day of mating was considered gestation day 0. Sprague-Dawley rats have a 21.5-day gestation period. The animals were housed in cages with free access to water and conventional laboratory chow. After birth, male pups were randomly assigned to control or treatment litters consisting of 12 pups each. The mothers were rotated daily among litters in an effort to maintain nutritional balance. Mothers were exposed to ADSS for 1 day at a time and then rotated to litters in filtered air for at least 2 days before being exposed again to ADSS. A subset of mothers rotated among litters were maintained continuously in filtered air. All pups were weaned at 21 days of age. Time points for immunohistochemistry and cell kinetics were 7, 14, 21, 50, and 100 days of age. At each time point, four animals from each group were examined for epithelial cell kinetics and three animals from each group were used for immunohistochemistry.

Exposure System

The sidestream smoke exposure system has been reported in detail elsewhere (28). Briefly, ADSS was generated by burning temperature- and humidity-conditioned Kentucky 1RF4 reference cigarettes (purchased from the Tobacco and Health Research Institute, Lexington, KY) in a smoking machine. ADSS was drawn into a glass and stainless steel Hinners-type exposure chamber housing the animals. The chamber atmosphere was monitored daily for CO, nicotine, and total suspended particulate matter (TSP). Rats were exposed to aged and diluted sidestream smoke (nicotine: 350 $\mu g/m^3$; TSP: 1 mg/m^3; CO: 6 ppm) as a surrogate for ETS 6 h a day, 5 days a week by whole-body exposure from birth until the day before sacrifice.

Tissue Preparation

Neonatal animals were anesthetized with sodium pentobarbital (1 mg/kg) and sacrificed by transection of the abdominal aorta. Lungs were fixed by intratracheal infusion at 20 cm water pressure for 30 min with 2% glutaraldehyde in cacodylate buffer (pH 7.4, 350 mOsm) for cell kinetics or with 1% paraformaldehyde for 1 h for immunohistochemistry. Fixed lung volumes were measured by the fluid displacement method of Scherle (29).

Cell Kinetics

Animals were intraperitoneally injected with 1 mCi/kg tritiated thymidine ([^{3}H]TdR) (specific activity: 6.7 Ci or 247.9 GBq per mmol) 1 h before sacrifice. Samples of glutaraldehyde-fixed lungs were sliced, and tissue blocks containing both distal and proximal bronchi were dehydrated in a graded series of ethanol before embedment in glycol-methacrylate. The sections were coated with photographic emulsion (50%) and developed after 6 wk. A cell was considered labeled if it had at least four silver grains over the nucleus (30). A minimum of 1,000 epithelial cells were counted per animal from two to four tissue blocks. Sections from the same block were cut at least 35 to 40 μm apart to assure that each slice contained distinct populations of nuclei. LI was expressed as the ratio of the number of cells labeled with [^{3}H]TdR to the total number of cells in the epithelial cell population of the airways.

Immunohistochemistry

Animals at the ages of 7, 14, 21, 50, and 100 days were examined immunohistochemically for the expression and distribution of cytochrome P450 isozyme 1A1. In addition, isozyme 2B, NADPH reductase, and Clara cell 10 kD (CC10) protein were investigated in the same animals. All lung tissues used for immunohistochemical localization were embedded in paraffin. We utilized the avidin-biotin peroxidase method as outlined by Hsu and associates (31). Briefly, the sections were deparaffinized in three changes of xylene for 5 min each and hydrated in decreasing concentrations of ethanol. Sections were then treated with 3% hydrogen peroxide in water to eliminate endogenous peroxidase and to unmask the antigenic sites. Bovine serum albumin (10%) in phosphate-buffered saline (PBS) containing 1.5% rat serum was then added to block nonspecific binding sites. The tissue was incubated with primary antibody at 4°C overnight. The polyclonal antibody used to identify cytochrome P450 isozyme 1A1 was raised in rabbits against purified rat liver enzyme (anti-1A1; Oxygene, Dallas, TX). CC10 antibody, a generous gift from G. Singh (University of Pittsburgh, Pittsburgh, PA) (32, 33), was made in rabbit against rat purified CC10; this antibody labels only bronchiolar epithelial cells. Anti-2B and anti-reductase, a generous gift from R. Philpot (NIEHS, Research Triangle Park, NC), were made in goat against rabbit purified lung antigens (34). The appropriate dilutions of the antibodies were as follows: anti-1A1, 1:25; all others,

1:5,000. As a control, primary antibody was substituted with PBS in each run to examine the tissue for nonspecific reaction. The ABC reagents were purchased in kit form from Vector Laboratories (Burlingame, CA).

Statistical Analysis

ANOVA and Duncan's multiple-range test were used to analyze differences in LIs between control and ADSS-exposed rats and for different age groups. A P value < 0.05 between the control and a treatment group was considered significant.

Results

Cell Kinetics

The cells that had taken up [^{3}H]TdR were generally dome-shaped, lacked cilia, and were evenly distributed throughout the airway epithelium. In both control and ADSS-exposed rats, none of the labeled cells were identified as ciliated cells. In terminal bronchioles, 49 of 1,000 epithelial cells in 7-day-old controls were labeled with [^{3}H]TdR. LIs in the terminal bronchioles of control animals decreased with age; by 100 days of age, none of the cells were labeled with [^{3}H]TdR (Figure 1). ADSS exposure significantly decreased the LIs in terminal bronchioles at 7 and 14 days of age ($P < 0.05$). Notable differences existed between ADSS-exposed and age-matched controls through 50 days of age, although significant differences in LIs between ADSS-exposed and control rats disappeared by 21 days of age.

Epithelium of the proximal bronchi had fewer labeled cells than that of terminal bronchioles in controls at all ages examined except 100 days (Figure 2). There was a statistically significant difference in LIs between proximal bronchi and distal airways ($P < 0.05$) at 7 days of age. As in the terminal bronchiole, LI in the proximal bronchi also decreased with age; however, unlike that at the terminal bronchiole, ADSS exposure did not significantly affect labeling at this level at any age examined. Fixed lung volumes were not significantly different between control and exposed animals at 7 (control: 1.195 ± 0.088 ml; ADSS exposed: 1.200 ± 0.304 ml), 14 (control: 2.117 ± 0.182 ml; ADSS exposed: 2.035 ± 0.262 ml), 21 (control: 2.97 ± 0.26 ml; ADSS exposed: 3.02 ± 0.11 ml), 50 (control: 10.11 ± 0.45 ml; ADSS exposed: 9.01 ± 0.65 ml), and 100 days of age (control: 12.33 ± 0.49 ml; ADSS exposed: 11.79 ± 0.19 ml).

Immunohistochemistry

Cytochrome P450 isozyme 1A1 protein expression. In 7-day-old controls (Figures 3A and 4A), the dome-shaped (Clara) cells lining the bronchioles were apically labeled; staining was observed to a lesser extent in type II cells. When staining of the proximal bronchi and distal airways was compared on the same section, proximal bronchi exhibited a greater frequency and intensity of staining than observed in epithelial cells of the distal airways. Both the number of cells stained and the intensity of staining for 1A1 antigen increased with age, until they reached the maximal level observed at 21 days of age (Figures 3B and 4B) compared with all other ages examined in this study. At 21 days of age, the bronchiolar epithelial cells in control animals were darkly stained, as were type II cells. Differences in the number and intensity of stained cells between proximal bronchi and distal airways were not evident at this age. By 50 and 100 days of age, the extent and intensity of staining were less obvious compared with 21 days of age in control animals (Figures 3C, 3D, 4C, and 4D). By 100 days, 1A1 antigen expression was

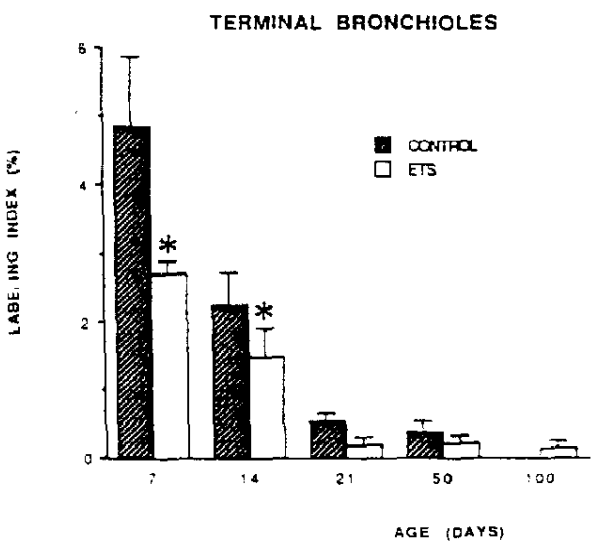

Figure 1. Developmental effects of ADSS exposure on labeling index of epithelial cells in the terminal bronchiole at 7, 14, 21, 50, and 100 days of age. A significant decrease (*) in the labeling index at 7 and 14 days of age was noted. A trend of decreased cell labeling index was noted through 50 days of age. Four animals were examined per age group.

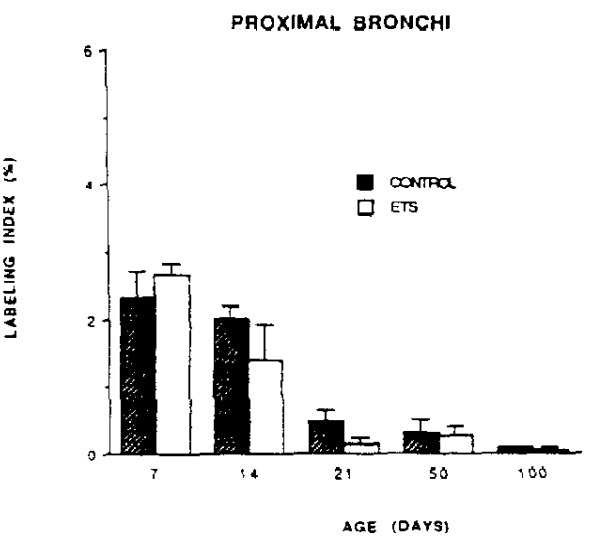

Figure 2. Developmental effects of ADSS exposure on labeling index of epithelial cells in the proximal bronchi at 7, 14, 21, 50, and 100 days of age. Four animals were examined per age group. No significant differences were noted between control and ADSS-exposed animals.

Figure 3. The immunohistochemical expression and distribution of cytochrome P450 isozyme 1A1 in rat proximal bronchi. 1A1 protein is located primarily in nonciliated bronchiolar epithelial (Clara) cells. ADSS exposure increases the expression of 1A1 antigen in proximal bronchi compared with that in age-matched controls. Note the striking difference in the expression of this protein at 50 and 100 days of exposure. A through D: Control rats; E through H: ADSS-exposed rats; A and E: 7 days; B and F: 21 days; C and G: 50 days; D and H: 100 days. Bar = 25 μm.

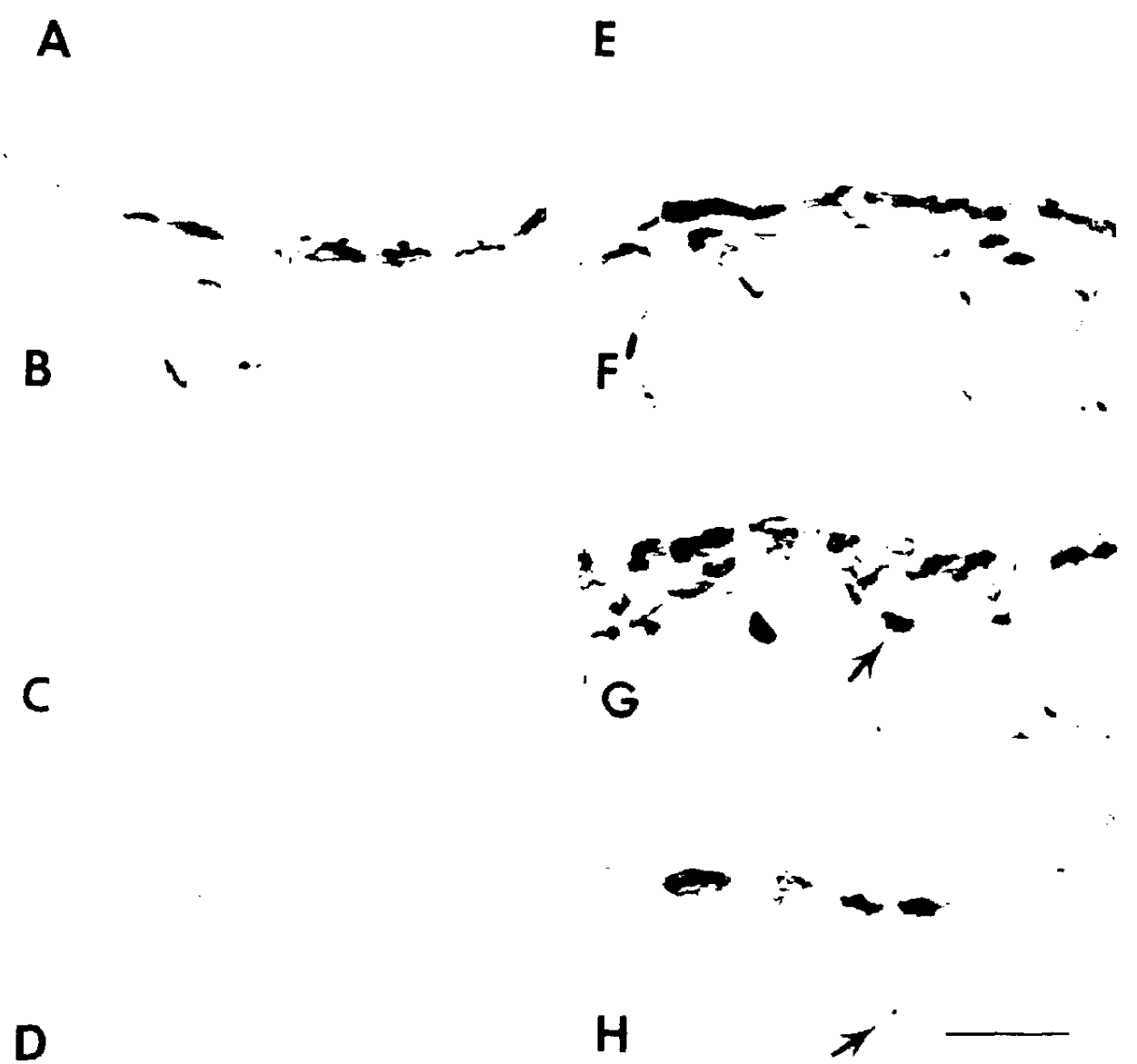

Figure 4. The immunohistochemical expression and distribution of cytochrome P450 isozyme 1A1 in rat terminal bronchioles. 1A1 protein is located primarily in nonciliated bronchiolar epithelial (Clara) cells, and to a lesser extent in type II cells (*arrows*). ADSS exposure increases the expression of 1A1 antigen in terminal bronchioles compared with that in age-matched controls. There is a striking difference in the expression of this protein at 50 and 100 days of exposure. A through D: Control rats; E through H: ADSS-exposed rats; A and E: 7 days; B and F: 21 days; C and G: 50 days; D and H: 100 days. Bar = 25 μm.

greater in dome-shaped (Clara) cells than in any other cell type in the lungs. In 50- and 100-day-old controls, the distribution and intensity for cytochrome P450 isozyme 1A1 were reduced as compared with controls at 21 days.

Compared with age-matched controls, ADSS exposure from birth significantly increased the expression and distribution of 1A1 antigen in Clara cells of proximal and distal airways and alveolar type II cells in the lung parenchyma from 7 through 100 days of age (Figures 3 and 4). This increase was much greater in bronchiolar epithelial cells than in type II cells with progressive exposure. The most striking difference was observed in animals at 50 and 100 days of age. ADSS exposure did not change the distribution of 1A1 antigen from one cell type to other types. In ADSS-exposed animals, the maximal expression of 1A1 antigen was at 50 days of age (Figures 3G and 4G).

Expression of cytochrome P450 isozyme 2B, NADPH reductase, and CC10 in Clara cells. Cytochrome P450 isozyme 2B and NADPH reductase are normally distributed in dome-shaped epithelial (Clara) cells of the airways and type II cells of the parenchyma, although not all cells contain these proteins. The expression of NADPH reductase (Figure 5) was augmented in bronchiolar epithelial cells by ADSS exposure at 21 and 50 days but not at 7 or 100 days of age. In contrast, ADSS did not change the cytochrome P450 isozyme 2B staining intensity or distribution (Figure 5) in airway epithelial cells at any of the ages examined in this study.

CC10 was distributed in the dome-shaped nonciliated epithelial (Clara) cells of the airways, but not all nonciliated cells were noted to have this protein. CC10 was in the apical portion of the Clara cell cytoplasm in rats 7 days of age and subsequently extended throughout the cytoplasm with greater age. ADSS exposure did not change either the staining intensity or the number of stained cells at the time points examined (Figure 5).

Discussion

The purpose of this study was to examine the effects of ADSS, as a surrogate of environmental tobacco smoke, on the postnatal development of the lung. This study addresses the impact of ADSS on differentiation of bronchiolar epithelium, one of the major target sites for inhaled pulmonary toxicants. ADSS exposure depressed cell kinetic activity in epithelial cells of terminal bronchioles but not in those of proximal bronchi. ADSS also induced the expression of cytochrome P450 1A1 and NADPH reductase in the Clara cells of both proximal bronchi and terminal bronchioles and, to a lesser extent, in type II cells of the parenchyma, but it did not change the expression of cytochrome P450 isozyme 2B or Clara cell secretory protein.

An interesting finding of this study was a distinct difference in the age-related decrease in the rate of cell division in proximal bronchi compared with terminal bronchioles during postnatal lung development in normal rats. LIs in proximal bronchi were consistently lower than in distal airways at every age except 100 days. If the decrease in epithelial cell kinetic activity is an indication of the status of differentiation, then these data suggest that the epithelium of proximal bronchi differentiate at a faster rate than epithelial cells in distal bronchioles. Other indicators of cell differentiation, such as cellular composition and secretory protein in proximal and distal airways, are also time dependent (35, 36). In a study of fetal rabbits, 6% of epithelial cells were labeled in animals of 28 days gestational age (23). The cell division rate (4.9%) in bronchioles of 7-day-old postnatal rats is about the same as that of fetal rabbits (23), but it is much higher than that of mature rats or postnatal mice (0.52%) (37). It is not clear from these other studies whether there exists any difference in the cell division rate between proximal bronchi and terminal bronchioles in mice or rabbits. Our findings indicate that high cell kinetic activity in normal prenatal animals continues throughout the early postnatal ages and then decreases dramatically to adulthood, when kinetic activity is very low.

ADSS exposure depressed cell division in distal airways but not in proximal bronchi at all ages examined. Because most lung cells respond to injury and other stimuli with increased cell proliferation (38), we expected to find an increase in LI at the early ages if various components in ADSS had injured epithelial cells. Since cell proliferation in the lungs of young animals is usually related to growth or to a low level of cell turnover, our finding implies that ADSS exposure may stimulate bronchiolar cell maturation and, therefore, bronchiolar development in the neonatal rat. However, since ADSS contains a wide range of compounds known to damage the epithelium of bronchioles, such as NO_2, another possible explanation for the observed decrease in LIs in the distal airways is that various components of ADSS inhibit the enzymes involved in DNA synthesis or block access to the four nucleotides, especially [^{3}H]TdR. Hyperoxia manifests toxicity in the mouse by inhibiting DNA synthesis in the lung (39). Studies with oxidant gases, such as hyperbaric oxygen and ozone, indicate that before weaning, animals are less sensitive to pulmonary injury than adults, although this response varies among species (40). Although it is not known whether there exists such a difference in rats, a threefold increase in bronchiolar LI was reported in 30-day-old rats following exposure to NO_2 (41). However, our study shows that the depression of cell kinetics after ADSS exposure is more obvious in young animals, which suggests that neonatal animals are more susceptible to ADSS than are animals of an older age. An additional explanation for the lowered LIs is that ADSS exposure either induces enzymes that degrade [^{3}H]TdR or damages feedback mechanisms that moderate DNA synthesis. We found that ADSS exposure did not affect LIs in proximal bronchi. This observation suggests that less mature cells, which are found predominantly in the distal airways, are more susceptible to injury from ADSS exposure. Furthermore, the difference in concentration of ADSS and other local factors, such as pH between proximal and distal airways, may contribute to this regional disparity in response to ADSS. The mechanisms causing the changes of LI in these neonates are not known, but obviously the toxic stress created by ADSS plays an important role.

Enzymatic activity associated with cytochrome P450 1A1 can be induced by a number of toxicants, including diesel exhaust (24) and cigarette smoke (25–27). These studies measured enzyme activity in whole-lung homogenates of mature animals. As a consequence, these studies could not determine which cell type(s) among more than 40 found in the

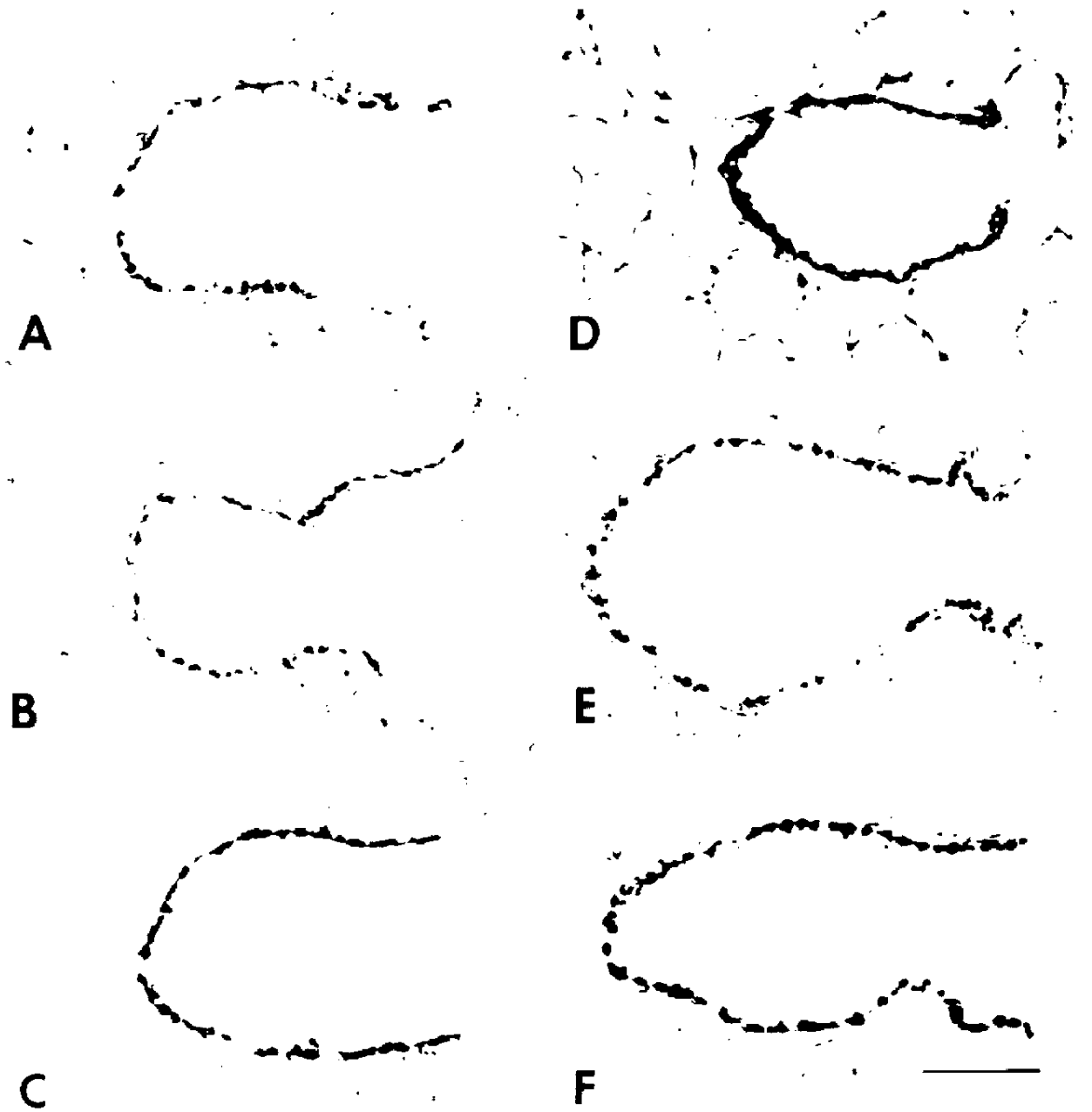

Figure 5. Immunohistochemical characterization of other Clara cell proteins in terminal bronchioles of rats at 50 days of age. NADPH reductase expression is increased by ADSS exposure (A and D). Cytochrome P450 2B (B and E) and Clara cell protein (CC10) (C and F) expression are not changed by ADSS exposure. A through C: control rats; D through F: ADSS-exposed rats. Bar = 100 μm.

lungs (11) respond to the toxicants or whether neonatal animals have the same responses as adults. This study was designed to examine the 1A1 cellular induction using well-characterized sidestream cigarette smoke in the rat lung during critical periods of postnatal development. ADSS exposure increased 1A1 protein expression in Clara cells of rats at all the ages examined but was most marked in older animals (50 and 100 days of age). In both proximal and distal airways, nonciliated bronchiolar epithelial cells showed an increased level of P450 1A1 protein after exposure to ADSS. This increase in protein expression can be traced to two sources: more cells that contain this protein and higher levels of the protein in those cells. This is consistent with the notion that P450 isozyme 1A1 can be induced in the lung by polyaromatic hydrocarbons. Some components of ADSS can increase the amount of P450 1A1 protein in cell types that al-

ready express it, such as Clara cells. For this reason, Clara cells have the highest level of O-methylated guanine after exposure to nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), an ADSS component bioactivated in part by P450 1A1 isozyme (42). A similar increase in P450 1A1 by ADSS exposure does not occur in endothelial cells of the blood vessels and occurs only minimally in type II cells of the parenchyma. This finding contrasts with other studies in rats using different inducers, such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (43) and 3-methylcholanthrene (44), in which P450 isozyme 1A1 is induced primarily in endothelial cells. It has also been shown in rabbits that TCDD increases 1A1 protein levels primarily in endothelial cells and to a lesser extent in Clara cells and type II cells (34). Another study using TCDD in rabbits found that P450 1A1 activity, as measured by O-deethylation of 7-ethoxyresorufin, was greatly increased in macrophages, Clara cells, and type II cells. The relative activity in endothelial cells was not measured (45). However, an important difference between these studies and ours was the manner in which inducers were given intraperitoneally, compared with the inhalation route in our study. Systemic routes of introducing 1A1 inducers stimulates the production of 1A1 in different lung cell populations than does exposure via inhalation. This finding implies that 1A1 induced in Clara cells by ADSS plays an important role in bioactivation of airborne procarcinogens in neonatal animals, whereas TCDD-induced 1A1 in endothelial cells can also be involved in bioactivation of systemically delivered compounds. The induction of 1A1 by ADSS may also differ from that by β-naphthoflavone (β-NF), since the aryl hydrocarbon hydroxylase activity (usually associated with 1A1) was found to be increased more in type II cells than in Clara cells isolated from β-NF–treated rats compared with controls (46).

It is well accepted that the bronchiolar tree has a proximal-to-distal wavelike developmental pattern (47). In most species, including rodents (36, 48, 49), primates (50), and probably humans (51), bronchiolar epithelial cells develop postnatally. This pattern is also true for marker proteins, such as the P450 monooxygenase system and CC10 (36), used to characterize Clara cells. Our study shows that, unlike other proteins, cytochrome P450 isozyme 1A1 increases with age postpartum, reaches the highest levels observed at 21 days, and then drops to very low levels in animals at 50 and 100 days of age. This pattern of loss of 1A1 protein during lung development may be part of the differentiation process for Clara cells. ADSS exposure from birth maintains the P450 1A1 protein levels even in adults, whereas P450 1A1 levels were noted to be low in control adult animals. Therefore, an alternative explanation for the increased distribution and expression of this protein could be that ADSS components actually delay the maturation of Clara cells. To test this possibility, we also investigated the effects of ADSS exposure on the expression of other Clara cell proteins (cytochrome P450 isozyme 2B, NADPH reductase, and CC10). ADSS exposure did not change the time course of the expression of P450 2B or CC10 even at 50 days of age, an age at which the difference in P450 isozyme 1A1 expression was greatest between ADSS-exposed and control animals. This finding suggests that ADSS exposure does not delay all aspects of Clara cell differentiation and that 1A1 development is not associated with the maturation of other proteins. NADPH reductase is present in postnatal rabbits as early as 2 days of age, well before P450 proteins are detectable (7 days) (15). NADPH reductase expression is increased by ADSS exposure at 21 and 50 days of age, which suggests that developmental expression for NADPH reductase is not closely linked to either 1A1 or 2B.

We conclude that ADSS exposure from birth alters the differentiation of bronchiolar epithelial cells. ADSS exposure accelerates the postnatal decrease of cell kinetic activity in epithelium of terminal bronchioles but not in epithelium of proximal bronchi. Exposure to ADSS from birth accelerates and maintains the expression of cytochrome P450 1A1 protein in Clara cells and type II cells and therefore may increase the capacity for bioactivation of some airborne procarcinogens during the development of the rat lung.

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